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US-PAT-NO: 6372499
DOCUMENT-IDENTIFIER: US 6372499 B1

TITLE: Polymeric complexes for the transfection of nucleic acids, with residues causing the destabilisation of cell membranes

DATE-ISSUED: April 16, 2002

INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/455; 424/486, 435/320.1, 435/325, 435/69.1

CLAIMS:

What is claimed is:

1. A complex comprised of at least one negatively charged nucleic acid and at least one positively charged polymeric conjugate with the bond therebetween being electrostatic in nature,

the polymeric conjugate containing a polylysine formed from monomers having free NH.sub.3.sup.+ groups,

at least 10% of free NH.sub.3.sup.+ groups of the said polylysine are substituted by residues which are protonated in a weakly acid medium causing destabilization of cell membranes,

and optionally at least one free NH.sub.3.sup.+ group of the said polylysine is substituted by a molecule with a recognition signal recognized by a cell membrane receptor,

with the proviso that all the free NH.sub.3.sup.+ groups of the said polysinc make up at least 30% of the number of monomers of the skeleton of the polymeric conjugate,

wherein said residues causing destabilization of cell membrane in a weakly acid medium belong to the family of quinolines of the formula: ##STR18##

in which R.sub.1 is hydrogen, R.sub.2 is --(CH.sub.2).sub.n --CO.sub.2 --H, X is hydrogen or chlorine and n is an integer from 1 to 10, wherein said recognition signal is selected from the group consisting of:

a) simple osides selected from the group consisting of .alpha. or .beta. conformers of 2-deoxy, 2-amino or 2deoxy, 2-acetamido neutral monosaccharides;

(g) Phosphorylated oligomannoside ##STR25##

(h) Oligosaccharide of the type of sulphated lactosamine of the formula ##STR26##

i. Lactose,

j. Fuc.alpha.2Gak.beta.3 (fuc.alpha.4) GlcNAc.beta.1Gal.beta.3Glc,

k. Fuc.alpha.4 (Ga.beta.3) GlcNAc.beta.3Gal.beta. and

l. Man.alpha.6-man.

6. The complex of claim 5 wherein the peptides are selected from the group consisting of

vasodilator intestinal polypeptide (VIP)

HSDAVFTDNYTRLRKQMAVKKYLNSILN-NH.sub.2 (SEQ ID No: 2)

antrial natriuretic polypeptide (ANP)

SLRRSSCFGGRMDRIGAQSGLGCNSFRY (SEQ ID No: 3)

lipocortin

HDMNKVLDL (SEQ ID No: 4)

bradykinin

RPPGFSPER (SEQ ID No: 5);

peptides of intergrins, peptide hormones and chemotactics factors.

7. The complex of claim 1 wherein the polymeric conjugate has the formula: ##STR27##

wherein

p is an integer from 15 to 900,

10 to 45% of the radical R being a residue with an imidazole nucleus,

10 to 90% of R being free NH.sub.3.sup.+ groups,

and optionally 0 to 45% of R being --NH--CO--(CHOH).sub.m --R.sub.1, m is an integer from 2 to 15, and R.sub.1 is hydrogen or alkyl of 1 to 15 carbon atoms.

8. The complex of claim 7 wherein R is a residue with an imidazole nucleus of the formula: ##STR28##

9. The complex of claim 7 wherein the polymeric conjugate has the following

formula: ##STR29##

wherein

p is an integer from 15 to 900,

10% to 45% of R is a residue having an imidazole nucleus and optionally a free NH.sub.3.sup.+, R has the formula: ##STR30##

30% to 90% of the number of R, having free NH.sub.3.sup.+, and 0 to 45% of R are substituted by a molecule which constitutes a recognition signal by a cell membrane receptor,

with the proviso that all the free NH.sub.3.sup.+ functions make up at least 30% of the number of monomer units of the polymeric skeleton of the above mentioned polymeric conjugate.

10. A complex according to claim 1 wherein the nucleic acid is selected from the group consisting of:

a) marker genes and

b) genes encoding a therapeutic protein.

11. Positively charged polymeric conjugate containing a polylysine formed from monomers having free NH.sub.3.sup.+ groups:

at least 10% of the free NH.sub.3.sup.+ groups of the said polylysine are substituted by residues which are protonated in a weakly acid medium causing destabilization of cell membranes,

and optionally some of the free NH.sub.3.sup.+ groups of the said polylysine can be substituted by a molecule with a recognition signal recognized by a cell membrane receptor,

with the proviso that all the free NH.sub.3.sup.+ groups of the said polylysine make up at least 30% of the number of monomers of the skeleton of the polymeric conjugate,

wherein said residues causing destabilization of cell membranes in a weakly acid medium belong:

to the family of quinolines of the formula ##STR31##

in which R.sub.1 is hydrogen, R.sub.2 is (CH.sub.2).sub.n --CO.sub.2 --H, X is hydrogen or chlorine and n is an integer from 1 to 10, wherein said recognition signal is selected from the group consisting of:

simple osides selected from the group consisting of .alpha. or .beta. conformers of 2-deoxy, of 2-amino or of 2-deoxy, 2-acetamido neutral monosaccharides; .alpha. or .beta. conformers of glycuronic acid derivatives of neutral monosaccharides; .alpha. or .beta. conformers of L-iduronic acid, of keto-deoxy-octonic acid, of M-acetyl-neuraminic acid, or of N-glycoloyl-neuraminic acid; and .alpha. or .beta. conformers of neutral 6-deoxy monosaccharides;

a disaccharide selected from the group consisting of lactose and mannopyranosyl.alpha.-6-mannopyranose,

and complex osides selected from the group consisting of Lewis.sup.a, Lewis.sup.b, Lewis.sup.z, oligomannosides and oligolactosamines, and peptides.

12. The positively charged polymeric conjugate according to claim 11 wherein the free NH.sub.3.sup.+ groups of the polylysine are substituted with a non-charged residue causing a reduction in the positive charge of the polymeric conjugate which facilitates salting out of the nucleic acids upon dissociation of the complex, said non-charged residue being a gluconyl.

13. The composition comprising the complex of claim 1 and an inert pharmaceutical carrier.

14. A method of transfecting cultured cells comprising incubating said cells in the presence of the composition of claim 13 under conditions wherein said composition enters said cells, and the nucleic acid comprised in the complex of said composition is released to transfect cultured cells.

15. The method of claim 14 wherein the cells are selected from the group consisting of

cells of hematopoietic strains;

dendritic cells;

liver cells;

skeletal muscle cells;

skin cells;

fibroblasts,

keratinocytes,

dendritic cells,

melanocytes;

cells of the vascular walls;

endothelial;

smooth muscle;

epithelial cells of the respiratory tract;

cells of the central nervous system;

cancerous cells; and

cells of the immune system.

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.alpha. or .beta. conformers of glycuronic acid derivatives of neutral monosaccharides; .alpha. or .beta. conformers of L-iduronic acid, of keto-deoxy-octonic acid, of N-acetyl neuraminic acid, or of N-glycoloyl-neuraminic acid; and .alpha. or .beta. conformers of neutral 6-deoxy monosaccharides;

b) a disaccharide selected from the group consisting of lactose and mannopyranosyl .alpha.-6-mannopyranose,

c) complex osides selected from the group consisting of Lewis.sup.a, Lewis.sup.b, Lewis.sup.x, oligomannosides and oligolactiosamines and

d) peptides.

2. The complex of claim 1 wherein said quinolines are selected from the group consisting of 7-chloro-4-(amino-1-methyl-butylamino)-quinoline, N.sup.4 -(7-chloro-4-quinolinyl)-1,4-pentanediamine, 8-(4-amino-1-methylbutylamino)-6methoxyquinoline (primaquine), N.sup.4 -(6-methoxy-8-qunolinyl)-1,4-pentanediamine, and pyridines selected from the group consisting of nicotinic acid and quinolenic acid and pterines.

3. The complex of claim 1 wherein the free NH.sub.3.sup.+ groups of the polylysine are substituted with a non-charged gluconyl residue causing a reduction in the positive charge of the polymeric conjugate which facilitates salting out of the nucleic acids upon dissociation of the complex.

4. The complex of claim 1 wherein said recognition-signal is a peptide chosen from the group consisting of

(a) anti-inflammatory peptides recognized by receptors of the vascular wall,

(b) ligand peptides of integrins,

(c) chemiotactic factors and

(d) peptide hormones.

5. The complex of claim 1 wherein:

the monosaccharide are selected from the group consisting of galactose, mannose, fucose, glucose, ribose, xylose and rhamnose and

the complex osides are selected from the group consisting of

(a) Asialo-oligoside of the type of triantennar lactosamine of ##STR19##

(b) Asialo-oligoside of the type of tetraanetennar lactosamin of the formula ##STR20##

(c) Lewis x of the formula ##STR21##

(d) Lewis x sialyl of the formula ##STR22##

(e) Sulphated Lewis x derivative (HNK1) of the formula ##STR23##

(f) Oligomannoside of the formula ##STR24##